

**PRELIMINARY AMENDMENT**  
**DIV of USSN 09/429,003**

**AMENDMENTS TO THE SPECIFICATION:**

**Please amend the specification as follows:**

**Page 1, after the title and before line 1,**

This Application is a Divisional of U.S. Application No. 09/429,003, filed October 29, 1999 (now allowed); which in turn is a Continuation of PCT/GB98/01261 filed April 30, 1998, which claims benefit of priority under 35 U.S.C. § 365 and § 120; t.——The disclosure of each of which PCT/GB98/01261—is incorporated herein by reference.

**FIELD OF THE INVENTION**

**Page 1, lines 5-8,**

**BACKGROUND OF THE INVENTION**

There are numerous examples of diagnostic methods that include physical, anatomical and behavioural examination and/or biochemical, electrical, or electromagnetic studies and/or assays.

**Page 5, lines 13-37,**

**SUMMARY OF THE INVENTION**

Thus, viewed from one aspect the present invention provides a method of preparing a gene transcript pattern probe kit for diagnosing or identifying a disease or condition or stage thereof in a prokaryotic or eukaryotic organism comprising at least the steps of:

- a) isolating mRNA from the tissue, cells or body fluid of a normal prokaryotic or eukaryotic organism (normal sample);
- b) isolating mRNA from the corresponding tissue, cells, or body fluid of an organism of step a) having the disease or condition of interest or a stage thereof (diseased sample);

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- c) separating the mRNA of steps a) and b), which may optionally be reverse transcribed to cDNA, by a non-sequence based separation technique;
- d) selecting two or more mRNA or cDNA species which are present at different levels in the normal and diseased samples;
- e) isolating the mRNA or cDNA species identified in step d);
- f) optionally reverse transcribing the mRNA of step d) or e) to cDNA, unless this has previously been performed in step c); and
- g) immobilizing the mRNA or cDNA probes of step e) or f) on one or more solid supports.

**Page 6, lines 1-11,**

DETAILED DESCRIPTION OF THE INVENTION

As used herein the disease or condition may be any condition, ailment, disease or reaction that leads to the relative increase or decrease in the activity of informative genes of any or all eukaryotic or prokaryotic organisms regardless of whether these changes have been caused by the influence of bacteria, virus, prions, parasites, fungi, radiation, natural or artificial toxins, drugs or allergens, including mental conditions due to stress, neurosis, psychosis or deteriorations due to the ageing of the organism, and conditions or diseases of unknown cause.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a standard diagnostic gene expression pattern in graphical form for a disease in Arabidopsis; and

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Figure 2 shows the extent of binding of cDNA isolated from *Picea abies* challenged with different types of stress to 7 different probes.

EXAMPLES

Example 1: Diagnosis of Alzheimer syndrome

A blood sample is collected from a patient suspected of suffering from the ailment. The sample is immediately preserved in liquid nitrogen to prevent degradation of the mRNA of the sample.

**Page 29, lines 18-26,**

**4.1.4 Adapter annealing**

5pmol/ $\mu$ l each of the two TaqI adapters and the two AseI adapters are pre-mixed.

TaqI adapter 1: GACGATGAGTCCGAC (SEQ ID NO:1)

TaqI adapter 2: CGGTCAGGACTCAT (SEQ ID NO:2)

AseI adapter 1: CTCGTAGACTGCGTACC (SEQ ID NO:3)

AseI adapter 2: TAGGTACGCAGTC (SEQ ID NO:4)

The mixture is heated for 2 min at 70°C and cooled down slowly to 30°C.

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**4.1.6 Preamplification**

5 $\mu$ l samples from the previous step are each mixed with 2.5  $\mu$ l amplification buffer (0.1M Tris-HCl, pH 8.3, 0.5M KCl, 15mM MgCl<sub>2</sub> and 0.1% (w/v) gelatin), 1Al (5ng) AseI preamplification primer, 1 $\mu$ l (30ng) TaqI preamplification primer, 1 $\mu$ l dNTP mix, 13.5 $\mu$ l water and 1 $\mu$ l (1U) TaqI DNA Polymerase.

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Asel preamplification primer: CTCGTAGACTGCGTACCTAAT (SEQ ID NO:5)

TaqI preamplification primer: GACGATGAGTCCTGACCGA (SEQ ID NO:6)

A temperature cycle of 94°C for 30 sec, 56°C for 30 sec and 72°C for 1 min is run for 25 cycles.

**4.1.7 Amplification**

5 $\mu$ l of the samples from the preamplification step are each mixed with 1 $\mu$ l Asel primer (Sng), 1p.1 TaqI primer (30ng), 0.2 $\mu$ l Taq Polymerase (1U), 4 $\mu$ l amplification buffer, 0.4 $\mu$ l dNTP mix and 8.4 $\mu$ l water. The primers are end labelled with y32P-ATP.

Asel amplification primer: GACTGCGTACCTAATNN (SEQ ID NO:7)

**Page 30, lines 1-2,**

TaqI amplification primer: GATGAGTCCTGACCGANN (SEQ ID NO:8) (N denotes any of the four deoxynucleotides)